

Discussion

Early eelgrass restoration efforts in the Chesapeake Bay involved transplanting adult eelgrass plants from healthy source beds to restoration locations. Averaging \$37,000 per acre not including monitoring (Fonseca, 1998), this and other restoration methods are both expensive and labor intensive and can damage donor beds. Despite some advantages to using adult plants (e.g. successful adult plants yield reproductive shoots during the following year's reproductive season, Orth, 2003), seed broadcasting appears to be a more efficient and cost effective restoration technique with the added benefit of having less impact on donor beds (Orth, 2000).

The results of the 2003-2004 restoration efforts have highlighted some of the obstacles to large-scale seed broadcasting. Seed storage represents the largest investment of time and resources, yet due to high seed mortality in the summer, relatively few viable seeds have been broadcast in the fall. Water quality plays a critical role in the survival of seedlings that have germinated. The results from each site vary greatly, so the discussion will be divided by site.

Site Selection

The most important step of any restoration project is selecting the proper location. Many previous restoration projects have suffered from improper siting (Harrison 1987; Fonseca 1992). The sites used for eelgrass restoration for this project were chosen using the DNR targeting system, which is designed to assess large areas of the Bay for their restoration potential. The model uses six layers of data to evaluate the suitability, ability and potential of a particular habitat to support SAV populations. After the targeting system identified areas, the sites selected underwent a two-year site selection process of test plantings and water quality monitoring. However, through test plantings and evaluation of 2003 and 2004 results, the Piney Point and Sage Point sites were found to exhibit less than ideal conditions. In additions, some factors that are difficult to include in the targeting system, like future water quality conditions, current velocity, and sediment type can have negative effects on seed establishment. Unfortunately, it is only through test plots and intensive monitoring that these factors can be evaluated. In 2006, DNR will increase seeding efforts in the sites that were most successful in 2004 and 2005.

Seed Collection

Two methods were used to count seeds, one for the spring seed bag method and one for fall seed broadcast method. The number of seeds dispersed using the spring seed bag method was determined shortly after collection by counting the total number seeds in four, 1 L replicate subsamples of reproductive material and multiplying the resulting seeds/L by the volume of seed material in each seed bag, and then the number of seed bags in a given plot.

The seed estimate for the fall seed broadcast method was made after all of the seeds had fallen from the reproductive shoots and were separated from the decaying reproductive material.

The first seed count method (used for spring seed bags) estimates the total number of seeds collected and included in each seed bag. As not all the seeds in every spathe can be expected to be viable, it may not accurately reflect the true number of viable seeds dispersed. The method used to enumerate seeds for the fall seed broadcasts also determines the viability of seeds. As such, it is likely to be more accurate. However, this method also has some sources of uncertainty. Because good seeds separate from bad seeds in water, it is necessary to drain all of the water from the seed slurry and completely mix the seed mixture before obtaining a representative sample. In addition, human error is a factor in both measuring samples out as well as the squeeze test for viability. When measuring aliquots, seeds are very sensitive to packing, creating a lot of variability between the 2 ml samples. During the squeeze test a seed is deemed viable or not viable based on physical robustness of the seed. There is considerable subjectivity in this determination as well. Efforts were made to keep the methods as uniform as possible, but because of the vast number of counts that are made it is not feasible to use the same staff member to conduct all counts. We have not been able to determine to what degree these sources of error affect our estimates.

In 2003, eelgrass seed harvesting was done using SCUBA and snorkeling gear to harvest reproductive shoots by hand. While this method was effective, the labor involved in these collections was very great, and only 2.3 million seeds were collected- ultimately limiting the size of potential restoration areas. In 2004, the use of a mechanical harvester allowed for the collection of 15.12 million seeds with roughly the same effort as 2003. In 2005, additional staff and boats further increased the efficiency of mechanical seed collection.

When using the mechanical harvester to collect eelgrass reproductive shoots, a number of steps were taken to minimize impact to the donor beds. As the reproductive shoots extend above the vegetative shoots, the depth of the cutting blades on the harvester were kept high enough above the sediment that the

rhizome mat and lower parts of the eelgrass plants were not disturbed. As a further precaution, harvesting took place over a large area to assure that sufficient seeds remain for bed maintenance (Granger, 2002). To confirm that there was no significant damage to eelgrass beds where reproductive shoots had been harvested, divers used SCUBA to survey the harvested beds 8 weeks (July 22, 2004) after the 2004 collection. Divers reported abundant, healthy eelgrass and quite a bit of flowering widgeon grass. There were no substantial differences in plant height, bed density, or apparent vigor of the plants themselves between the harvested and unharvested beds. In addition, aerial photography taken on June 19 and July 6, 2004 confirmed that the areas that were harvested in May were still densely vegetated (VIMS; 2004 field observations and aerial photography accessible: http://www.vims.edu/bio/sav/2004_obs.html#vims071304).

Test plantings

Test plots for each of the sites were planted in November of 2004, and surveyed in spring 2005. The test plots for the Sage Point had an 81% initial survival rate, but lost all of the above ground biomass before our August sampling. Cherryfield Point yielded a 31% initial survival rate, and also lost all biomass before August. St. George Island had an 86% initial survival rate, but unlike Sage Point and Cherryfield Point, 3% of the plants remained at the site in November. For 2006 efforts, a much higher proportion of seeds will be placed at the St. Georges Island location to attempt to build on this success.

The Piney Point seeding and seed bag site was located adjacent to the RKK Engineers eelgrass restoration site that was planted with adult plants. This allows for a side-by-side comparison of the adult plants and seedling. Of the eelgrass planted in 2003, after 6 months, 27% of eelgrass survived, and at 12 months less than 10% of all adult eelgrass plants remained.

Seed Viability

Storing the spring-harvested seeds through the summer is one of the most difficult aspects of this project. Each year there has been a substantial loss of seeds during seed storage, ultimately decreasing the number of viable seeds at the end of the storage process and reducing the acreage of SAV restored. Two million three hundred thousand seeds were collected in 2003, of the half that were stored at Piney Point through the summer, only 250,000 of these were viable and used for fall broadcast. Harvest efforts in 2004 collected 15.12 million seeds. However, only 7% of these (1,058,400 seeds) were deemed viable in the fall. The 2005 harvest collected 12.4 million seeds total. After distributing about half of the seeds in spring seed bags, 109.5 liters containing approximately 7,446,000 seeds remained at Piney Point. Unfortunately, only 2,527,000 seeds were viable at the end of the seed processing/storage procedure. After the 2004

season, biologists from VIMS and DNR attempted to identify potential problems with the seed transport and separation and holding/storage process. The lack of basic research on seed physiology made identifying specific problems very difficult. In 2005, seed storage experiments were set up at St. Mary's College, VIMS, and MD-DNR to test the impact of the following parameters: flow, aeration, salinity, and stirring. When the results of these experiments are analyzed, appropriate modifications will be made to the seeds processing and storage procedure to be applied to the 2006 seed collection.

Seed Bags/Seed Dispersal

In May, 2004, seed bags were deployed across 20 acres, with approximately 2.4 million seeds distributed. In the fall, 262,000 seeds were machine broadcast in a 1 acre plot. The estimated number of plants resulting from the spring seeding was 7,193, and the estimate for the fall was 147. Two factors could have contributed to the difference in the results; variance in number of seeds dispersed, and the time of year the seeds were dispersed. Assuming the seeds were the same, the difference in the number of seeds dispersed could explain the difference in recruitment. Regardless of the reason for the variance observed, spring seeding clearly was the most effective method for seed distribution in 2004.

Except for Piney Point, where no plants were observed, all of these sites had much greater success with the spring seed bag dispersal. Since the same seed bag material was used for Piney Point, this site appears to not have the restoration potential of the others, and will not be used in 2006. For fall seed broadcast, the recruitment was much lower due to poor seed survival during storage.

The results for the number of plants generated for each method was one of the most important components of this experiment. Data from this project will be used by DNR and other organizations to guide future large-scale restoration efforts. In looking at the raw numbers, it states a very clear case for using seed bags for future restoration. Both cost per seed distributed and cost per recruited eelgrass shoot was less expensive than fall broadcasting.

These results were compiled using all available data from the 2005 plants resulting from 2004 seeding efforts. One of the major factors in the lower costs was the lack of viable seeds the end of the summer. Seed storage has been one of the more difficult obstacles in this project. Each year, refinements have been made to increase seed viability, but the percentage of viable seeds per seed collected is still very low. DNR has been working with VIMS to make further improvements to this phase of the project. DNR will make modifications to the seed storage in 2006 to minimize the loss of viable seeds.

Eelgrass Survival related to water quality

Eelgrass seed distributions in 2004 resulted in the successful establishment of seedlings at each site in May, 2005. Clearly, seeding areas of the Potomac River could be an effective method for initiating eelgrass growth. However, almost all adult test plot plants and seedlings completely disappeared in the summer of 2005. If conditions were ideal and the plants had simply undergone summer defoliation, we would have expected to see those plants again during the November survey, during their fall growing period. This was not the case. Very few plants were seen during the November survey, which suggests that the plants died rather than underwent a seasonal defoliation.

To determine the cause for the near complete loss of adult plants, water quality data from the continuous monitoring stations, mainstem stations, and the water quality mapping cruises were analyzed to detect trends or spikes in water temperature and turbidity data that may explain these results. A number of studies have shown that decreased light availability affects eelgrass survival (Philips et al. 1978; Kemp et al. 1983; Dennison and Albert 1986; Twilley et al. 1985). Eelgrass requires between 6 and 8 hours of photosynthetic saturating irradiance per day to survive (Dennison and Alberte, 1985). Although it is not well documented how many days healthy plants can survive elevated turbidity and decreased light availability, it is not likely that the recruited seedlings or adult plants could survive the prolonged periods of high turbidity such as those reflected by the continuous monitor data. When water clarity data for 2003, 2004 and 2005 are compared to the 20-year record, the values are below the mean each year, with 2003 being the year with the worst water clarity (Fig. 19).

Turbidity values are one measure we have to determine light availability in the Potomac during our study period. Using the EPA requirement of 22% of surface irradiance for healthy SAV growth, and an application depth of 1.0 meter, a turbidity value of 5.38 NTU's was determined as the water clarity target for the mesohaline portion of the Potomac River.

The data reported here reflect the results of 2004 seeding efforts. Germination of these seeds took place in the fall of 2004, and those seedlings were subject to fall 2004 water quality upon germination. Keeping in mind the importance of light, when we look closely at the turbidity conditions occurring between our three sets of surveys, it is evident that there were episodes of severely elevated turbidity (turbidity higher than the 5.38 NTU threshold) at both of the continuous monitoring stations. At the Piney Point Station, turbidity levels were above the threshold for 34 days, with a majority of those days between May 1 and June 15, 2005. The water quality mapping data show that turbidity levels exceeded the threshold for the June and July cruises at each site. For the Sage

Point site, the turbidity levels exceeded the threshold for 26% of the year, all of which fell during the growing season. The water quality mapping data also show that turbidity levels exceeded the threshold in the months of June and July. The 2005 results showed that turbidity exceeded for 7% of the growing season at Piney Point, and 17% at Sage Point, but the water quality mapping data show that turbidity levels were below the threshold at each site for the entire summer.

Total Suspended Solids (TSS) and chlorophyll (calculated from fluorescence) concentrations are the two primary contributors to turbidity throughout Chesapeake Bay. In order to determine which parameter contributed most to light attenuation in the Potomac, correlation values were determined between turbidity and chlorophyll from the 2004 and 2005 continuous monitoring datasets. At the Piney Point station in 2004 and 2005, the Pearson correlation coefficients were 0.08 ($P < 0.0001$, $N = 16972$) and 0.005 ($P < 0.0001$, $N = 18441$, respectively). At the Sage Point station, 2004 and 2005 had Pearson correlation coefficients of 0.02 ($P < 0.0001$, $N = 17447$), and 0.0001 ($P < 0.0001$, $N = 19524$, respectively). These regression analyses indicate a weak correlation between chlorophyll and turbidity at both stations for both years. This weak correlation suggests that suspended solids are the primary contributor to the high turbidity in the Potomac River.

Temperature is the other water quality parameter that directly affects eelgrass survival. Eelgrass in the Chesapeake Bay is near the southernmost extent of its distribution on the east coast of the United States. Eelgrass growth slows and defoliation occurs at temperatures above 25⁰ C (Moore et al 1996 and 1997). Compounding this effect, when temperatures increase above just 20⁰ C, epiphyte loading increases substantially (Dr. Walter Boynton, personal communication). To look at potential temperature effects during this study, a maximum threshold for eelgrass survival of 30⁰ C was adopted. A comparison of the Piney Point and Sage Point sites shows that the temperature data were nearly identical in 2004, with both stations showing the temperatures below 30⁰ C for the entire season. In 2005, the Piney Point site recorded a temperature above 30⁰ C for 2.6% of the season and the Sage Point site for 3.4% of the season (Fig. 15 & 16). Although it is not well documented how many days healthy plants can tolerate these elevated temperatures, the fact that these instances of elevated turbidity coincide with elevated temperatures are the likely reason that most recruited seedlings and adult plants did not survive the summer of 2005.

In surveys done by VIMS at the end of the 2005 summer, there was a widespread defoliation of eelgrass beds in the Virginia portion of the Chesapeake Bay. Eelgrass typically undergoes an annual, summer defoliation, with detached leaves forming large floating wracks that end up on beaches around the bay (Orth, personal communication). This normally takes place in late June or early July. In 2005, however, the die-off was much more severe, with several areas that

had been vegetated for many years suffering a complete loss of above-ground biomass. In Virginia, the eelgrass loss is suspected to be due to a combination of higher than normal summer water temperatures, low winds, and lower than normal light levels (Orth, personal communication).

Cost Comparison

The large seed loss during storage (80%) is responsible for the significantly higher costs per seed and per acre using the fall seed broadcast method. If 50% of the total seeds retained throughout the processing and storage procedure, a total of 1,871,000 viable seeds would have been available for broadcast on the Potomac River. With the additional viable seeds and same total costs, the cost per seed would be reduced from \$0.34 to \$.04 and the cost per acre would drop from \$67,085 to \$7,157. This is a reasonable expectation, since VIMS retained 80% of total seeds as viable in 2005 (Orth, personal communication).

The high costs of processing associated with the seed broadcast method combined with the seed losses during storage make it significantly more expensive than dispersing seeds using the spring seed bag method. In order for fall seed broadcasting to achieve the same seed cost as the seed bag method (\$0.02/seed and \$4,473/acre) 2,992,000 seeds would have to be broadcast on the Potomac of the total 15.12 million seeds collected. In order for this to occur, seed viability would need to be increased significantly and a larger proportion of the total viable seeds would have to be allocated for the Potomac River than in previous years.

The recruitment success of each method was determined by dividing the total number of seeds dispersed by the number of successfully recruited plants. However, there was considerable difficulty in determining the number of viable seeds, so this analysis is largely speculative. The spring seed bag method yielded 7,193 seedling spread across all spring seed bag locations. A total of 2,400,000 seeds were dispersed using this method. Therefore, the recruitment success for the seed bag process was 0.30%. The fall seed broadcast method yielded 147 seedlings, requiring 262,000 seeds to achieve a 0.06% recruitment rate.

The total cost for each method was divided by the total number of successfully recruited seedlings to determine cost per successfully recruited seedling. Each seedling (7,193) successfully recruited using the spring seed bag method cost \$1.70. Each seedling for the fall seed dispersal method is \$363.89. At this point in the study, spring seed bags are a much more cost effective restoration technique than fall seeding. However, other restoration efforts using the fall seed broadcast method in Maryland (DNR) and Virginia (VIMS) resulted in recruitment rates ranging from .5 to 14% in 1999 and 4.3 to 13.8 in 2000. If DNR recruitment rate improved to be similar those observed at VIMS, the cost per

successfully recruited plant would be \$40.83-\$1.46 according to the 1999 rates and \$4.75-\$1.48 according to 2004 rates. This would bring the two techniques much closer in terms of cost effectiveness.

A spring seed bag project conducted in 2004 in VA (VIMS) resulted in a recruitment rate of 1.3%. Initial restoration efforts using the BuDSS in the Peconic Estuary, NY yielded 7% recruitment (Pickerell, 2003). If DNR recruitment rates for the seed bag method ranged from 1.3-4.0%, the cost per recruited plant would drop to \$0.39-\$0.13. The projected cost of \$0.39 per plant at a 1.3% recruitment rate would still be more cost efficient than the cost per plant using the seed broadcast with a 14% recruitment rate. Regardless of which method is used, it is clear that we must work to improve recruitment rates if this project is to have the potential for truly large-scale restoration.

Mitigation Plantings

The final report from RKK engineers indicated some possible reasons for the failure of the adult shoots. In some of the planting unit grid locations no plant material could be found, but planting anchors were located. This would indicate that some of the plant losses were due to poor installation. In other instances, root and rhizome material along with dead leaves were located anchored by the skewer. These rhizomes themselves were found to be dead and decaying. These observations indicate that some failure was due to plants not surviving the process of packing, shipping, holding or installation. For the 6 month survey, about half of the plants that were counted as not surviving appeared to have been dead prior to planting, and half appeared to be the result of poor installation.

Observations of the sago pondweed grids indicated similar reasons for failure. Half of the non-surviving plants appeared to have died after installation while the other half appeared to have been installed incorrectly. Of course, the major difference between sago and eelgrass plots is that almost all of the sago pondweed installed were counted as non-surviving. Widgeon grass PUs were counted as non-surviving with less frequency than Sago, however the reasons appeared to be roughly the same. Since DNR was not involved in the planting process, it is difficult to quantify if these plants failed for the reasons above, or the losses were primarily due to unfavorable water quality conditions.